

## Cytotoxic Activity and G1 Cell Cycle Arrest of a Dienynone from *Echinacea pallida*

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### Abstract

In the present study, a further investigation of the cytotoxic activity of an acetylenic constituent of *Echinacea pallida* roots, namely, pentadeca-(8Z,13Z)-dien-11-yn-2-one, was performed, revealing a concentration-dependent cytotoxicity on several human cancer cell lines, including leukemia (Jurkat and HL-60), breast carcinoma (MCF-7), and melanoma (MeWo) cells. As part of its mechanism of action, the ability of this constituent to arrest the cell cycle in the G1 phase was demonstrated on HL-60 cells. Furthermore, a stability test of the target compound over 72 h was carried out, indicating that the cytotoxic activity can be attributed mainly to the genuine, not oxidized, molecule.

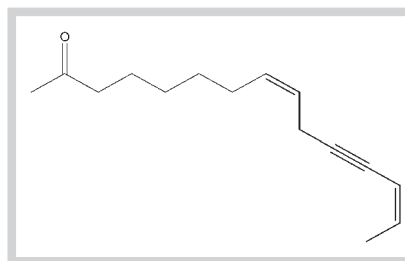
### Key words

*Echinacea pallida* (Asteraceae) · acetylenes · polyenes · cell cycle · cytotoxicity · cancer

Medicinally used *Echinacea* species (*E. pallida*, *E. angustifolia*, and *E. purpurea*, family Asteraceae) are among the most widely used medicinal plants, mainly for their immunomodulatory, anti-inflammatory and antioxidant properties [1,2]. Moreover, *Echinacea* preparations are often used by cancer patients, in addition to classical antineoplastic therapy, because of their ability to increase immune system activity [3,4].

*n*-Hexane extracts from the roots of the three above-mentioned *Echinacea* species have demonstrated *in vitro* anticancer activity on human cancer cell lines and *E. pallida* extracts have shown the greatest potency [5]. Furthermore, a bioassay-guided fractionation study has indicated that polyacetylenes and polyenes, which are present in high concentration in *E. pallida* lipophilic root extracts [6–8] but not detected in those from *E. purpurea* and *E. angustifolia* [9], are responsible for this activity [10]. In particular, pentadeca-(8Z,13Z)-dien-11-yn-2-one (● Fig. 1) has been revealed to be a potent cytotoxic compound that is able to induce apoptotic cell death at low micromolar concentrations in human cancer cell lines (pancreatic MIA PaCa-2 and colonic COLO320) [10]. This compound has also been demonstrated to inhibit P-glycoprotein efflux activity [11] and to have a good potential for oral bioavailability [10].

The present work focused on the evaluation of the cytotoxic activity of pentadeca-(8Z,13Z)-dien-11-yn-2-one on different human cancer cell lines and the possible involvement of cell cycle arrest in its mechanism of action. Moreover, a comparison of the cytotoxic effects of the natural and the synthesized compound was performed.



**Fig. 1** Chemical structure of pentadeca-(8Z,13Z)-dien-11-yn-2-one.

**Table 1** IC<sub>50</sub> values of pentadeca-(8Z,13Z)-dien-11-yn-2-one and positive control on human cancer cell lines after 72 h of exposure.

Compound	IC <sub>50</sub> value (μM) ± SEM			
	Jurkat	MCF-7	HL-60	MeWo
Dienynone	2.1 ± 0.6	2.5 ± 0.7	21.3 ± 0.8	28.6 ± 2.3
Positive control <sup>a</sup>	0.9 ± 0.2	3.4 ± 0.5	0.8 ± 0.3	2.6 ± 0.9

Note: Data (mean ± SEM) are from three experiments carried out in triplicate; <sup>a</sup> 5-Fluorouracil was the positive control for MCF-7 and HL-60; cisplatin was the positive control for Jurkat and MeWo

The results showed that pentadeca-(8Z,13Z)-dien-11-yn-2-one exerts concentration-dependent cytotoxic activity on all the tested human cancer cell lines (leukemia Jurkat and HL-60, breast carcinoma MCF-7 and melanoma MeWo), as indicated by the IC<sub>50</sub> values reported in ● Table 1. The 10-fold greater sensitivity of Jurkat and MCF-7 cells compared with HL-60 and MeWo cells may be due to the different drug-resistance properties of cell lines or cell line subclones used in this study. A similar difference was previously reported for this compound between pancreatic Mia PaCa-2 (a particularly apoptotic-resistant line) and colonic COLO320 cells [5,10]. In several cases, the cytotoxicity of pentadeca-(8Z,13Z)-dien-11-yn-2-one was of the same order of magnitude as that of anticancer drugs currently used in therapy, such as doxorubicin on MCF-7 cells [12] and cisplatin on Jurkat cells [13]. The anticancer drugs 5-fluorouracil and cisplatin were used as internal positive controls and their IC<sub>50</sub> values are reported in ● Table 1. It is noteworthy that in a previous study, this compound demonstrated no significant cytotoxicity up to 100 μM on human non-cancer cells [10].

With regard to effects on the cell cycle, in HL-60 cells, pentadeca-(8Z,13Z)-dien-11-yn-2-one induced arrest in the G1 phase after 72 h of exposure, as reported in ● Table 2: at 20 μM (IC<sub>50</sub> value) 83% of the cell population was in the G1 phase, while at 40 μM (2 × IC<sub>50</sub> value) 90% of cells were in the G1 phase. Similar data were obtained with the other cell lines (data not shown). These results indicate that G1 cell cycle arrest is one of the main mechanisms responsible for the cytotoxic activity of pentadeca-(8Z,13Z)-dien-11-yn-2-one; moreover, they strengthen the evidence that such a mechanism plays an important role in the anticancer activity of natural acetylenic compounds, as reported for falcarinol [14,15], panaxydol [16], and other similar compounds [17].

Due to the well-known difficulty in purifying this acetylenic compound from *E. pallida* roots [6,7], whose extracts contain many other constituents of similar polarity, and owing to the need for higher amounts of this constituent for biological assays, a total synthesis of this secondary metabolite was recently carried out [18]. In the present study, the cytotoxic activity of both natural (isolated and purified from *E. pallida* roots) and synthetic

**Table 2** Cell cycle distribution of HL-60 cells after treatment with negative control or pentadeca-(8Z,13Z)-dien-11-yn-2-one for 72 h.

Compound	Percentage of HL-60 cells arrested in:		
	G1 phase	S phase	G2 phase
Negative control	69.7 ± 1.9%	12.6 ± 2.3%	12.7 ± 1.2%
Dienynone (20 µM) <sup>a</sup>	82.9 ± 1.3%	8.9 ± 0.8%	4.6 ± 1.6%
Dienynone (40 µM) <sup>b</sup>	90.3 ± 1.1%	5.9 ± 1.1%	3.3 ± 1.0%

Note: Data (mean ± SEM) are from three experiments carried out in triplicate. <sup>a</sup> IC<sub>50</sub> value; <sup>b</sup> 2×IC<sub>50</sub> value

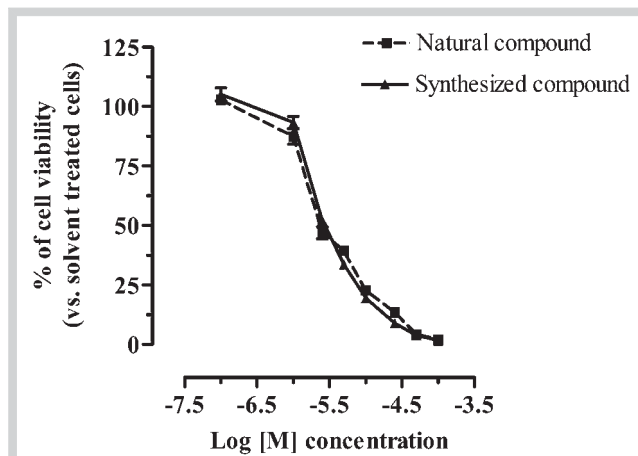
pentadeca-(8Z,13Z)-dien-11-yn-2-one was compared in order to investigate whether the synthetic approach may affect the anti-cancer activity of this molecule. The results showed no differences in cytotoxic activity between the natural and synthesized compounds, which exhibited the same potency on the Jurkat cell line (● Fig. 2), thus confirming the accuracy of the total synthesis of this secondary metabolite.

The results of the stability study of pentadeca-(8Z,13Z)-dien-11-yn-2-one in the culture medium are shown in ● Fig. 3. As described in the literature [6,19], the oxidation of the authentic compounds isolated from *E. pallida* roots proceeds via an intermediate hydroperoxide, which is then reduced to the corresponding alcohol. The results of the present study indicated that the oxidation kinetics of pentadeca-(8Z,13Z)-dien-11-yn-2-one is quite slow, since the area of the hydroperoxide intermediate was about 25% after 72 h of exposure. The final product of the oxidation process, i.e. the hydroxylated derivative, was not observed in the HPLC after 72 h of exposure. Considering that during the first 24 h of exposure, the area of the hydroperoxide intermediate is below 15%, the observed cytotoxic activity can be attributed mainly to the genuine, not oxidized, compound. More detailed investigations of the oxidation kinetics of authentic polyacetylenes and polyenes isolated from *E. pallida* roots are currently in progress.

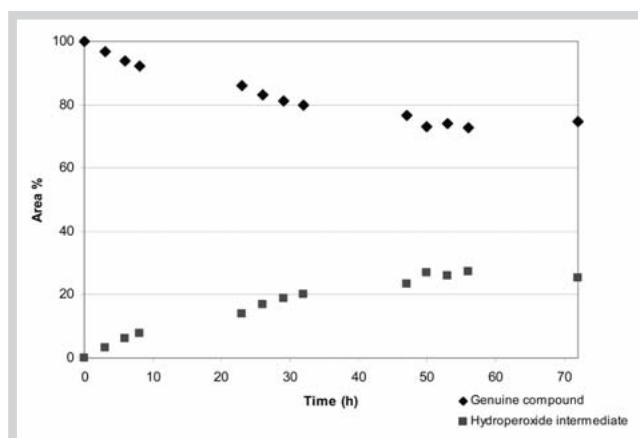
In conclusion, the cytotoxic activity of pentadeca-(8Z,13Z)-dien-11-yn-2-one against several human cancer cell lines has been demonstrated, and the arrest of the cell cycle in the G1 phase has been shown to be involved in its mechanism of action. Further studies are in progress to elucidate possible cellular targets (e.g., COX or LOX isoforms, cannabinoid or PPAR receptors, histone deacetylase) and molecular pathways (as specific apoptotic molecules) involved in the cytotoxic activity of this secondary metabolite. Possible synergism with chemotherapeutic drugs is under investigation as well.

## Materials and Methods

Extraction and purification of pentadeca-(8Z,13Z)-dien-11-yn-2-one from *E. pallida* roots was carried out as previously described [6,7]. Synthesis of the compound was performed in agreement with the literature [18]. The UV, IR, NMR, and MS data of the isolated and synthesized compounds matched well with those in the literature [6,18]. The degree of purity of the tested compound was 98% as determined by RP-HPLC [7]. Considering the well-known low chemical stability of *E. pallida* polyacetylenes and polyenes [6], DMSO stock solutions of pentadeca-(8Z,13Z)-dien-11-yn-2-one were stored at low temperature (−80 °C), protected



**Fig. 2** Cytotoxic profile of natural and synthesized pentadeca-(8Z,13Z)-dien-11-yn-2-one on the Jurkat human leukemia cancer cell line after 72 h of exposure.



**Fig. 3** Stability study of pentadeca-(8Z,13Z)-dien-11-yn-2-one as monitored by RP-HPLC-DAD for 72 h.

from light and kept under argon atmosphere to prevent any chemical oxidation until required for the biological assays. Under these storage conditions, the compound was found to be stable. To check the stability of the tested compound under the applied experimental conditions, a stability study was performed by preparing a 100 mM solution of pentadeca-(8Z,13Z)-dien-11-yn-2-one in DMSO, which was further diluted to 100 µM concentration with the culture medium. The resulting solution was incubated for a set interval at 37 °C and sampled at fixed time intervals for the RP-HPLC-DAD analysis [7].

All cell lines were obtained from the American Type Culture Collection (ATCC) and cultured under the following conditions: RPMI 1640 medium supplemented with L-glutamine (2 mM), 10% fetal bovine serum, 50 IU/mL penicillin, and 50 µg/mL streptomycin (Sigma-Aldrich). Cells were maintained at 37 °C in a humidified incubator under 5% CO<sub>2</sub>.

Cell viability was assessed using the cell proliferation reagent WST-1 (Roche) as previously described [10].

Cell cycle analysis by flow cytometry was performed as previously described [20]. Briefly, 10<sup>6</sup> cells were plated in a 100-mm

culture dish and incubated for 48–72 h. Then, cells were fixed with 90% ethanol and incubated with a staining solution containing RNase A (30 µg/mL) and propidium iodide (50 µg/mL) in phosphate-citrate buffer (pH 7.2). Cellular DNA content was analyzed by flow cytometry using a Becton Dickinson laser-based flow cytometer. At least 20 000 cells were used for each analysis. Differences among compound treatments were evaluated by the one-way ANOVA test followed by the Newman-Keuls post-test. A *p* value ≤ 0.05 was considered to be significant.

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## References

- Gertsch J. Immunomodulatory lipids in plants: plant fatty acid amides and the human endocannabinoid system. *Planta Med* 2008; 74: 638–650
- Woelkart K, Linde K, Bauer R. *Echinacea* for preventing and treating the common cold. *Planta Med* 2008; 74: 633–637
- Richardson MA, Sanders T, Palmer JL, Greisinger A, Singletary SE. Complementary/alternative medicine use in a comprehensive cancer center and the implications for oncology. *J Clin Oncol* 2000; 18: 2505–2514
- Dy GK, Bekele L, Hanson LJ, Furth A, Mandrekar S, Sloan JA, Adjei AA. Complementary and alternative medicine use by patients enrolled onto phase I clinical trials. *J Clin Oncol* 2004; 22: 4810–4815
- Chicca A, Adinolfi B, Martinotti E, Fogli S, Breschi MC, Pellati F, Benvenuti S, Nieri P. Cytotoxic effects of *Echinacea* root hexanic extracts on human cancer cell lines. *J Ethnopharmacol* 2007; 110: 148–153
- Pellati F, Calò S, Benvenuti S, Adinolfi B, Nieri P, Melegari M. Isolation and structure elucidation of cytotoxic polyacetylenes and polyenes from *Echinacea pallida*. *Phytochemistry* 2006; 67: 1359–1364
- Pellati F, Calò S, Benvenuti S. High-performance liquid chromatography analysis of polyacetylenes and polyenes in *Echinacea pallida* by using a monolithic reversed-phase silica column. *J Chromatogr A* 2007; 1149: 56–65
- Morandi S, Pellati F, Ori C, Adinolfi B, Nieri P, Benvenuti S, Prati F. Isolation, structure elucidation and total synthesis of a cytotoxic dienyne from *Echinacea pallida*. *Org Biomol Chem* 2008; 6: 4333–4339
- Barnes J, Anderson LA, Gibbons S, Phillipson JD. *Echinacea* species (*Echinacea angustifolia* (DC.) Hell., *Echinacea pallida* (Nutt.) Nutt., *Echinacea purpurea* (L.) Moench): a review of their chemistry, pharmacology and clinical properties. *J Pharm Pharmacol* 2005; 57: 929–954
- Chicca A, Pellati F, Adinolfi B, Matthias A, Massarelli I, Benvenuti S, Martinotti E, Bianucci AM, Bone K, Lehmann R, Nieri P. Cytotoxic activity of polyacetylenes and polyenes isolated from roots of *Echinacea pallida*. *Br J Pharmacol* 2008; 153: 879–885
- Romiti N, Pellati F, Nieri P, Benvenuti S, Adinolfi B, Chieli E. P-Glycoprotein inhibitory activity of lipophilic constituents of *Echinacea pallida* roots in a human proximal tubular cell line. *Planta Med* 2008; 74: 264–266
- Tassone P, Tagliaferri P, Perricelli A, Blotta S, Quaresima B, Martelli ML, Goel A, Barbieri V, Costanzo F, Boland CR, Venuta S. BRCA1 expression modulates chemosensitivity of BRCA1-defective HCC1937 human breast cancer cells. *Br J Cancer* 2003; 88: 1285–1291
- Mariggiò MA, Cafaggi S, Ottone M, Parodi B, Vannozzi MO, Mandys V, Viale M. Inhibition of cell growth, induction of apoptosis and mechanism of action of the novel platinum compound cis-diaminechloro-[2-(diethylamino) ethyl 4-amino-benzoate, N(4)]-chloride platinum (II) monohydrochloride monohydrate. *Invest New Drugs* 2004; 22: 3–16
- Bernart MW, Cardellina 2nd JH, Balaschak MS, Alexander MR, Shoemaker RH, Boyd MR. Cytotoxic falcarinol oxylipins from *Dendropanax arboreus*. *J Nat Prod* 1996; 59: 748–753
- Kuo YC, Lin YL, Huang CP, Shu JW, Tsai WJ. A tumor cell growth inhibitor from *Saposhnikovia divaricata*. *Cancer Invest* 2002; 20: 955–964
- Moon J, Yu SJ, Kim HS, Sohn J. Induction of G(1) cell cycle arrest and p27 (KIP1) increase by panaxydol isolated from *Panax ginseng*. *Biochem Pharmacol* 2000; 59: 1109–1116
- Park C, Kim GY, Kim GD, Lee WH, Cheong JH, Kim ND, Bae SJ, Jung JH, Choi YH. Suppression of U937 human monocytic leukemia cell growth by dideoxypetrosynol A, a polyacetylene from the sponge *Petrosia* sp., via induction of Cdk inhibitor p16 and down-regulation of pRB phosphorylation. *Oncol Rep* 2006; 16: 171–176
- Morandi S, Pellati F, Benvenuti S, Prati F. Total synthesis of a dienyne from *Echinacea pallida*. *Tetrahedron* 2008; 64: 6324–6328
- Bauer R, Khan IA, Wagner H. TLC and HPLC analysis of *Echinacea pallida* and *E. angustifolia* roots. *Planta Med* 1988; 54: 426–430
- Lee SK, Heo YH, Steele VE, Pezzuto JM. Induction of apoptosis by 1,4-phenylenebis(methylene) selenocyanate in cultured human colon cancer cells. *Anticancer Res* 2002; 22: 97–102

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